control of the rtTA-dependent promoter. In offspring containing both transgenes, that marker will only be produced in cells expressing rtTA, and only in the presence of tetracycline derivatives. As a result, the only cells in the offspring which synthesize the marker are those cells in which the gene mutated by the provirus is expressed. These cells, depending on the nature of the marker, may then be detected and, if desired, separated from the remaining cells using standard techniques. The marker may be any reporter of gene expression. Such reporters include, without limitation, the bacterial *lacZ* gene (An et al., Mol. Cell. Biol. **2**:1628-1632 (1982)), green fluorescent protein, wavelength variations of green fluorescent protein (Heim et al., Proc. Natl. Acad. Sci. USA **91**:12501-12504)), luciferase (de Wet et al., Mol. Cell. Biol. **7**:725-737 (1987)), and chloramphenicol acetyltransferase (CAT) (Gorman et al., Mol. Cell. Biol. **2**:1044-1051 (1982)).

The MAGEKO process facilitates conditional ablation of cell lineages expressing mutant genes. The use of the rtTA construct facilitates the ability to conditionally ablate cell lineages expressing mutant genes. Cell ablation studies are instrumental in assigning function to entire cell lineages, as has been demonstrated in several instances (Breitman et al., Science **238**:1563-1565 (1987); Behringer et al., Genes Dev. **2**:453-461 (1988); Landel et al., Genes Dev. **2**:1168-1178 (1988); Breitman et al., Development **106**:457-463 (1989); Heyman et al., Proc. Natl. Acad. Sci. USA **86**:2698-2702 (1989); Borrelli et al., Nature **339**:538-540 (1989); Breitman et al., Mol. Cell. Biol. **10**:474-479 (1990); Kunes and Steller, Genes Dev. **5**:970-983 (1991); Moffat et al., Development **114**:681-687 (1992); Nirenberg and Cepko, J. Neurosci. **13**:3238-3251 (1993); and Dzierzak et al., Intern. Immunol. **5**:975-984 (1993)). The retroviral vectors of the present invention are designed to utilize this powerful approach.

According to this aspect of the invention, conditional cell ablation is accomplished through a binary transgenic mouse system. In this system, a mouse that contains the "weapon" transgene in a silent form is mated to a mouse that expresses the activator. In

the offspring that inherit both transgenes, the "weapon" is activated, and it exerts its killing effects only in cells expressing the activator. In the context of the rtTA system, mice expressing rtTA under the control of the endogenous mouse gene promoter synthesize rtTA only in cells expressing the mutant gene (Figs. 3 and 4). These mice are mated with mice carrying conditionally produced "cell ablation factors" which are themselves synthesized only in the presence of both rtTA and tetracycline derivatives. Offspring containing both transgenes are subjected to cell ablation studies following administration of tetracycline derivatives and resultant destruction of cells expressing the gene with the retroviral insertion. Examination of these offspring provides a functional characterization of the ablated cell lineage.

Conditionally produced "cell ablation factors" useful in the invention include, but are not limited to, wild-type and mutant toxins (Borrelli et al., Nature **339**:538-540 (1989); Frankel et al., Mol. Cell. Biol. **9**:415-420 (1989); and Frankel et al., Mol. Cell. Biol. **10**:6257-6263 (1990)), wild-type and mutant herpes simplex virus thymidine kinases (HSV-tk) (Salomon et al., Mol. Cell. Biol. **15**:5322-5328 (1995); and Black et al., Proc. Natl. Acad. Sci. USA **93**:3525-3529 (1996)), and apoptotic proteins such as the *Drosophila reaper* gene product (White et al., Science **271**:805-807 (1996)). If an HSV-tk gene is utilized, gancyclovir, in addition to tetracycline derivatives, is administered to trigger cell killing. In another example, conditionally produced β-galactosidase may also be used to facilitate cell ablation, as shown for various cell types in the nervous system (Nirenberg and Cepko, J. Neurosci. **13**:3238-3251 (1993)).

Use of MAGEKO for temporal and spatial phenotypic analysis of disrupted genes. Use of the methods of the invention and, for example, the rtTA construct, also facilitates the temporal and spatial characterization of the phenotypes of disrupted genes. In many instances, especially if the insertional mutation in the homozygotic state is lethal or results in a phenotype interfering with further analysis (Copp, Trends Genet. **11**:87-93 (1995)), it is preferable to inactivate a gene of interest in an animal in a temporal and

spatial manner. In the present invention, this is accomplished through the use of mosaic animals derived from a mixture of ES cells, some of which are heterozygotic and some of which are homozygotic for mutations in the gene of interest. In these mosaic animals, the heterozygotic cells rescue those cells which are homozygotic, as has been generally demonstrated previously (Nagy and Rossant, J. Clin. Invest. **97**:1360-1365 (1996); and Robb et al., EMBO J. **15**:4123-4129 (1996)), and this leads to the generation of mosaics.

According to this aspect of the invention, mosaic mice are generated from homozygotic mutant ES cells in the gene of interest with mutant ES cells containing the identical proviral insertion in only one of the two alleles of the same gene. The heterozygotic cells (derived from animals generated as described above for the conditional ablation technique) also contain conditionally produced "cell ablation factors." These factors are synthesized only in the presence of both rtTA and tetracycline derivatives, and rtTA, in turn, is produced only in cells expressing the gene with the retroviral insertion (Figs. 3 and 4).

Administration of tetracycline derivatives to mosaic animals leads to the specific obliteration of heterozygotic cells in which the mutant gene is expressed, due to the presence of the "ablation factors" in those cells only. As a result, the cell population of an animal expressing the mutant gene will be exclusively composed of homozygotic mutant cells. Under these conditions, the phenotype associated with the gene of interest may be assessed. This approach is useful for the phenotypic analysis of mutants, particularly when generation of adult mice is compromised in the homozygotic state.

Use of the MAGEKO process for conditional tissue-specific gene inactivation. In some instances, temporal and spatial phenotypic analysis of a disrupted gene may not be adequate to assign gene function. To address this problem, a different but complementary approach, termed conditional tissue-specific gene inactivation, may be employed. According to this approach, a gene of interest is inactivated, when desired, in the cells in which it is expressed. This general technique has been previously used to